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10/811,353	03/26/2004	Paul B. McCray JR.	290.00670120	1559

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MUETING, RAASCH & GEBHARDT, P.A.
P.O. BOX 581415
MINNEAPOLIS, MN 55458

EXAMINER	
SHEN, WU CHENG WINSTON	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 11/30/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/811,353	MCCRAY ET AL.	
	Examiner	Art Unit	
	Wu-Cheng Winston Shen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-30 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-30 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application 10/811,353 filed on March 26, 2004 is a CIP of PCT/US03/17577 filed on 06/04/2003 claims the benefit of 60/386,064 filed on 06/04/2002.

Status of claims: Claims 1-30 are pending and currently under examination.

Claim Rejection - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1-30 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling to the extent for a method to introduce a selected nucleic acid sequence into an mammalian airway epithelial cell with a pseudotyped lentivirus *in vitro*, does not reasonably provide enablement for a method to introduce a selected nucleic acid sequence into an airway epithelial cell with any other pseudotyped retrovirus *in vivo* encompassed by the claims.

It is noted that claim 1 encompasses a method to introduce a selected nucleic acid sequences into a mammalian airway epithelial cell *in vitro* as well as *in vivo*. Because the specification contemplates on introduction of a selected nucleic acid sequences into a mammalian airway epithelial cell *in vivo* for therapeutic uses, the scope of claim 1 encompasses the scope of claim 19 --- a method to reduce or eliminate symptoms of cystic fibrosis in a mammal.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The nature of the invention: The nature of the invention is a method to introduce a selected nucleic acid sequence into an airway epithelial cell, preferentially to reduce or eliminate symptoms of cystic fibrosis in a mammal, comprising: contacting the airway epithelial cell with

a pseudotyped retrovirus, wherein the pseudotyped retrovirus comprises a glycoprotein in which a portion of an *O*-glycosylation region within the glycoprotein has been deleted and a retroviral capsid comprising the selected nucleic acid sequence, that comprises preferentially a nucleic acid sequence that encodes a cystic fibrosis transmembrane regulator protein.

The breadth of the claims: The breadth of claims 1-18, 19-30 encompass (i) a method to introduce any selected nucleic acid sequence into an airway epithelial cell of any organism, *in vitro* or *in vivo*, comprising: contacting the airway epithelial cell with a pseudotyped retrovirus, wherein the pseudotyped retrovirus comprises any glycoprotein in which a portion of an *O*-glycosylation region within the glycoprotein has been deleted, and any retroviral capsid comprising the selected nucleic acid sequence (claim 1), and (ii) a method to reduce or eliminate symptoms of cystic fibrosis in any mammal comprising: contacting an airway epithelial cell of the mammal, *in vitro or in vivo*, with any pseudotyped retrovirus that comprises any glycoprotein in which any portion of an *O*-glycosylation region within the glycoprotein has been deleted and any retroviral capsid that comprises a nucleic acid sequence that encodes a cystic fibrosis transmembrane regulator protein (claim 19).

The state of the prior art: McCray reviewed the difficulties of gene therapy in treating cystic fibrosis and stated that, most gene therapy approaches in cystic fibrosis involve the delivery of the vector to the lungs by instillation or as an aerosol. Thus the delivery vehicle is applied to the apical surface of the cells. Delivery via the vascular system might also be possible. Whatever the mechanism used, scientists recognize the respiratory epithelium as one of the most challenging targets for the practical application of gene therapy. Many different cell

types make up this complex epithelium, and the key types that must be targeted remain unknown. Since the pathology of cystic fibrosis is primarily confined to the airways, the cells of the conducting airways rather than the alveoli are the main therapeutic targets. However, the airway epithelium is in constant contact with the environment and has evolved a vast array of mechanisms and responses to keep microbes and other environmental challenges, including the vectors of gene transfer, from entering the host. McCray further stated, although many vector systems work in the laboratory, translation of these results into successful clinical trials has proven much more challenging. Difficulties include delivery of the vector to the cell, lack of persistent gene expression in targeted cells, and immune responses to viral gene products, transgenes, or cells targeted by the vectors. *Furthermore, mice with deletions of the CFTR gene or with common human CFTR mutations do not develop lung disease like people. There is not, therefore, a good animal model in which to test new therapies* (McCray, Cystic fibrosis: Difficulties of gene therapy. *Lancet* 358 Suppl: S19, 2001).

Traditional gene therapy vectors have demonstrated limited utility for treatment of chronic lung diseases such as cystic fibrosis (CF). Kobinger et al. described a vector based on a Filovirus envelope protein-pseudotyped HIV vector, which we chose after systematically evaluating multiple strategies. The vector efficiently transduces intact airway epithelium from the apical surface, as demonstrated in both *in vitro* and in mouse system *in vivo*. This shows the potential of pseudotyping in expanding the utility of lentiviral vectors. Pseudotyped lentiviral vectors may hold promise for the treatment of CF (See abstract, Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nat Biotechnol.* 19(3): 225-30, 2001).

Wang et al. investigated apical barriers to airway epithelial cell gene transfer with amphotropic retroviral vectors stated, gene transfer to airway epithelia with amphotropic pseudotyped retroviral vectors is inefficient following apical vector application. To better understand this inefficiency, Wang et al. localized the expression of Pit2, the amphotropic receptor, in polarized human airway epithelia. Pit2 was expressed on both the apical and basolateral surfaces of the cells, suggesting that factors other than receptor abundance may limit apical gene transfer efficiency. Binding studies performed with radiolabeled amphotropic MuLV suggested that the apically applied virus binds to Pit2. Hypothetical barriers to retroviral gene transfer include the apical glycocalyx and other secreted products of epithelia. Wang et al. demonstrated that sialic acid, keratan sulfate and collagen type V are present on the apical surface of well-differentiated human airway epithelia. While enzyme treatment reduced the abundance of these components, the treatment also decreased the transepithelial resistance to approximately 35% of the controls, suggesting that the epithelial integrity was impaired. To attain an airway epithelial culture with a modified apical surface and intact epithelial integrity, we utilized 100 mM 2-deoxy-D-glucose, a glycosylation inhibitor, to prevent the glycocalyx from reforming following enzyme treatment. This approach allowed the resistance, but not the apical glycocalyx to recover. Despite this physical modification of the cell surface, the amphotropic retroviral vector failed to transduce airway epithelia following apical application. The results by Wang et al. suggest that factors other than apical receptor abundance and the glycocalyx inhibit amphotropic retroviral gene transfer in human airway epithelia (Wang et al., Apical barriers to airway epithelial cell gene transfer with amphotropic retroviral vectors. *Gene Ther.* 9 (14): 922-31, 2002).

Sinn et al. stated that, the practical application of gene therapy as a treatment for cystic fibrosis is limited by poor gene transfer efficiency with vectors applied to the apical surface of airway epithelia. Folate receptor alpha (FR alpha), a glycosylphosphatidylinositol-linked surface protein, was reported to be a cellular receptor for the filoviruses. Sinn et al. found that polarized human airway epithelia expressed abundant FR alpha on their apical surface. In an attempt to target these apical receptors, Sinn et al. pseudotyped feline immunodeficiency virus (FIV)-based vectors by using envelope glycoproteins (GPs) from the filoviruses Marburg virus and Ebola virus. Importantly, primary cultures of well-differentiated human airway epithelia were transduced when filovirus GP-pseudotyped FIV was applied to the apical surface. Furthermore, by deleting a heavily O-glycosylated extracellular domain of the Ebola GP, Sinn et al. improved the titer of concentrated vector several fold. To investigate the folate receptor dependence of gene transfer with the filovirus pseudotypes, Sinn et al. compared gene transfer efficiency in immortalized airway epithelium cell lines and primary cultures. By utilizing phosphatidylinositol-specific phospholipase C (PI-PLC) treatment and FR alpha-blocking antibodies, Sinn et al. demonstrated FR alpha-dependent and -independent entry by filovirus glycoprotein-pseudotyped FIV-based vectors in airway epithelia. Of particular interest, entry independent of FR alpha was observed in primary cultures of human airway epithelia. Understanding viral vector binding and entry pathways is fundamental for developing cystic fibrosis gene therapy applications (See abstract, Sinn et al., Lentivirus vectors pseudotyped with filoviral envelope glycoproteins transduce airway epithelia from the apical surface independently of folate receptor alpha. *J Virol.* 77(10): 5902-10, 2003).

Verhoeyen et al. reviewed the different approaches explored to upgrade lentiviral vectors, aiming at developing vectors suitable for *in vivo* gene delivery and stated, vectors derived from retroviridae offer particularly flexible properties in gene transfer applications given the numerous possible associations of various viral surface glycoproteins (determining cell tropism) with different types of retroviral cores (determining genome replication and integration). Lentiviral vectors should be preferred gene delivery vehicles over vectors derived from onco-retroviruses such as murine leukemia viruses (MLVs) that cannot transduce non-proliferating target cells. Generating lentiviral vectors pseudotyped with different viral glycoproteins (GPs) may modulate the physicochemical properties of the vectors, their interaction with the host immune system and their host range. There are however important gene transfer restrictions to some non-proliferative tissues or cell types and recent studies have shown that progenitor hematopoietic stem cells in G_0, non-activated primary blood lymphocytes or monocytes were not transducible by lentiviral vectors. Moreover, lentiviral vectors that have the capacity to deliver transgenes into specific tissues are expected to be of great value for various gene transfer applications in vivo. Several innovative approaches have been explored to overcome such problems that have given rise to novel concepts in the field and have provided promising results in preliminary evaluations *in vivo* (See abstract, Verhoeyen et al., Surface-engineering of lentiviral vectors. *J Gene Med.* 6 Suppl 1:S83-94, 2004).

Sanders reviewed virus entry and gene therapy stated, replacing the native viral envelope protein on the surface of a retrovirus or lentivirus with the glycoprotein of a foreign enveloped virus, a process called pseudotyping, can expand the set of potential target cells for a viral vector or can restrict entry to specific cells. The Ebola virus glycoprotein, because of its evolutionary

origins and the route of viral entry promoted by it, possesses distinct advantages in forming the outer shell of such pseudotyped retroviruses for gene therapy applications. Studies of the transduction of human airway epithelia by lentivirus pseudotyped with a modified Ebola virus glycoprotein from which the region of O- glycosylation has been removed have demonstrated that such recombinant viruses possess particular promise for the treatment of cystic fibrosis. The result highlights the synergism between basic studies of virus entry and gene therapy advances (See abstract, Sanders, Ebola virus glycoproteins: guidance devices for targeting gene therapy vectors. *Expert Opin Biol Ther.* 4 (3): 329-36, 2004). With regard to retrovirus and lentivirus, Sanders further stated, *oncoretroviruses, such as the commonly employed murine leukaemia viruses (MuLVs), do not generally transducer (integrate the genetic material that carry into) non-dividing cells. This limits their utility in many in vivo applications.* Lentiviruses, such as the human immunodeficiency virus type 1 (HIV-1) and feline immunodeficiency virus (FIV), are capable of transducing non-dividing cells and, therefore, have become for many the preferred retrovial vectors (See last paragraph, left column, page 330, Sanders, 2004).

With regard to routes of administration for delivery of pseudotyped retrovirus to airway epithelial cells, claimed invention encompasses a method to introduce any selected nucleic acid into airway epithelial cells (claim 1), and a method comprising contacting an airway epithelial cell of any mammal with any pseudotyped retrovirus (claim 19). Thus, the breadth of claims 1, 19 and their dependent claims 2-18, 20-30, encompasses any route of administration to deliver nucleic acid encoding any polypeptide to a tissue of any mammal, which results in the expression of the nucleic acid in the airway epithelial cells in the mammal. Practicing the claimed invention as broadly as claimed would require administration by any medically accepted means for

introducing the therapeutic directly or indirectly into a tissue *in vitro* or *in vivo*, including but not limited to injections (e.g., intraperitoneal, intravenous, intraarterial, intramuscular, transendocardial, subcutaneous, intracranial or catheter); oral ingestion; intranasal or topical administration; and the like. The choice of a particular route of administration for a given composition comprising a given nucleic acid to a tissue of a given mammal may not be applicable to any other route of administration for any other composition comprising any other given nucleic acid to any tissue of any other mammal.

While progress has been made in recent years for *in vivo* gene transfer, vector targeting *in vivo* to desired sites has continued to be unpredictable and inefficient for the past decade. This statement is supported by numerous teachings available in the art. For example, Miller et al. (Miller and Vile, Targeted vectors for gene therapy, *FASEB J.* 9(2): 190-9, 1995) reviewed the types of vectors available for *in vivo* gene therapy, including retroviral, adenoviral, liposomal, and molecular conjugates, and conclude that "for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances...targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Deonarain, Ligand-targeted receptor-mediated vectors for gene delivery, *Exp. Opin. Ther. Patents* 8(1): 53-69, 1998; Ashley Publications Ltd. ISSN 1354-3776) reviewed ligand-targeted receptor mediated vectors, and indicated that one of the biggest problems hampering successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time" (page 53, first paragraph). Deonarain reviewed techniques under experimentation in the art which showed promise, but which are even

less efficient than viral gene delivery (see page 65, first paragraph under Conclusion section). Verma et al. (Verma and Somia, Gene therapy -- promises, problems and prospects, *Nature* 389: 239-42, 1997) reviewed various vectors known in the art for use in gene therapy and the problems that are associated with each. Verma clearly indicated that resolution to vector targeting problems had not been achieved in the art (see entire article). Verma discussed the role of the immune system in inhibiting the efficient targeting of viral vectors such that efficient expression is not achieved (see page 239 and 2nd and 3rd column of page 242). Crystal (Crystal, Transfer of genes to humans: early lessons and obstacles to success, *Science* 270: 404-10, 1995) also reviewed various vectors known in the art and indicated, "among the design hurdles for all vectors are the need to increase the efficiency of gene transfer, to increase target specificity and to enable the transferred gene to be regulated" (page 409). As an alternative, Pouton et al. (Pouton and Seymour, Key issues in non-viral gene delivery, *Adv Drug Deliv Rev.* 46(1-3): 187-203, 2001) reviewed the issues in non-viral gene delivery and stated "direct injection of gene medicines into target tissue represents a far simpler task than targeting delivery to a specific tissue from the systemic circulation". See last full sentence on page 188, right column, and section 2.1. Pouton et al. added that there were "no systems yet available for efficient tissue targeting following systemic delivery." (See page 189, first sentence of section 2.2.). More recently, Read et al (Read et al., Barriers to gene delivery using synthetic vectors, *Adv Genet.* 53: 19-46, 2005) stated after the time the invention was filed that the "lack of suitable vectors for the delivery of nucleic acids... represents a major hurdle to their continued development and therapeutic application" (see abstract, sentence bridging pages 19 and 20. Problem areas included obtaining persistence in the circulation, gaining access to target cells, and distinguishing

target cells from non-target cells. See e.g. page 22). Finally, Dobson (Dobson, Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. *Gene Ther.* 13(4): 283-7, 2006) reviewed the development of non-viral transfection agents, as an alternative, for gene delivery stated "While magnetic targeting appears to hold significant potential for gene therapy, there are still major obstacles to employing this technique in the clinic. Perhaps, the problem that is most difficult to overcome is, as with magnetic targeting for drug delivery, that of scale-up." (See Prospects on page 286).

The predictability or lack thereof in the art: As stated in the proceeding section, there is lack of predictability in the art regarding a method of transducing airway epithelial cells of any mammal *in vitro* or *in vivo*. In this regard, not all retroviral vectors can be used for transduction of airway epithelial cells. With regard to the difference in mammal defective in cystic fibrosis transmembrane regulator protein (CFTR), mice with deletions of the CFTR gene or with common human CFTR mutations do not develop lung disease like people. There is not, therefore, a good animal model in which to test new therapies for a method to reduce or eliminate symptoms of cystic fibrosis. Other difficulties resulting in the lack of predictability include delivery of the vector to the cell, lack of persistent gene expression in targeted cells, and immune responses to viral gene products, transgenes, or cells targeted by the vectors.

The amount of direction or guidance: The specification provides general information regarding viral vectors, such as retroviral vectors, cystic fibrosis, airway epithelial cells, and (pages 1-3). The specification also provides general information regarding pseudotyped

retrovirus, envelope glycoproteins, and their uses in gene therapy for delivery of selected nucleic acid sequences (pages 14-22).

The presence or absence of working example: The specification provides working examples on preparation and reporter gene, comprising *LacZ* or GFP, transfer *in vitro,* comprising cultured human airway epithelial cells, 293T cells, and isolated rabbit tracheal segment, by pseudotyped viral particles having Feline Immunodeficiency Virus (FIV) capsid proteins and Vesicular Stomatitis Virus G protein (VSV-G), Marburg Virus (MRB) or Ross River Virus (RRV) glycoprotein (See example II), modified Ebola Virus glycoproteins and viral particles containing the modified Ebola Virus glycoproteins (Example III), use of pseudotyped viral particles to transducer airway epithelia in cultured cells *in vitro* (example IV). The specification fails to provide any *in vivo* working example or specific guidance regarding how the examples performed *in vitro* can be modified and applied to the *in vivo* condition when an mammal, rather than a cultured cell derived from the mammal, is under consideration.

In view of the state of the art, the unpredictability in the art, and the lack of specific guidance and working examples in the specification, one of skill in the art would have to perform undue experimentation to make and use the claimed invention as recited in claims 1-26.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

3. Claims 6 and 23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "Ebola virus glycoprotein lacks amino acids 309-489" fails to set forth the metes and bounds to particularly point out and distinctly claim the subject matter which applicant regards as the invention because there are multiple Ebola virus glycoproteins GP_0, GP_1, GP_2 and it's unclear what would be the amino acids 309-489 of these glycoproteins. Applicants are advised to provide SEQ ID No in this regard.

Priority Dates of Claims

4. Claims 5-6 and 22-23 benefit the priority date of 60/386,064 filed on 06/04/2002.

Claims 1-4, 7-21 and 24-30 benefit the priority date of 60/353,221 filed on 10/26/2001 and 60/356,436 filed on 10/26/2001.

It is noted that claims 5-6 and 22-23 fail to benefit the priority date of 60/353,221 filed on 10/26/2001 or 60/356,436 filed on 10/26/2001 because the provisional applications 60/353,221 and 60/356,436 fail to disclose (i) Ebola virus glycoprotein (claims 5, 22) (ii) Ebola virus glycoprotein lacks amino acid 309-489 (claims 6, 23). More detailed clarification is provided below.

With regard to provisional applications 60/353,221 and 60/356,436 filed on 10/26/2001, the provisional applications 60/353,221 and 60/356,436 disclosed novel filoviral glycoprotein-pseudotyped lentiviruses are provided. The viruses comprise a lentiviral capsid and a viral

envelope further comprising a lipid bilayer and a functional filoviral glycoprotein. In one embodiment the lentivirus is a feline immunodeficiency virus (FIV). In an alternate embodiment the viral glycoprotein is a Marburg virus glycoprotein. Preferably the Marburg virus glycoprotein has a mutation in the C-terminal portion of the amino acid sequence that results in a higher titer production of the pseudotyped virus. Methods of using the inventive pseudotyped lentiviruses for introducing nucleotide sequences into target cells are also provided (See abstracts of the provisional applications 60/353,221 and 60/356,436). More specifically, the mutation(s) in the C-terminal portion of the amino acid sequence disclosed in the provisional application 60/356,436 are C671A or Y679 stop mutation. The Y679 stop mutation reads on the limitation "a portion of an-O-glycoprotein region within the glycoprotein has been deleted" recited in claim 1 of instant application 10/811,353.

Claim Rejection - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 5 and 22 are rejected under 35 U.S.C. 102(b) as being anticipated by Kobinger et al. (Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia *in vivo*. *Nat Biotechnol.* 19(3): 225-30, 2001).

Claims 1-3, 7-20 and 23-30 are rejected under 35 U.S.C. 102(a) as being anticipated by Kobinger et al. (Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia *in vivo*. *Nat Biotechnol.* 19(3): 225-30, 2001).

Kobinger et al. teach that traditional gene therapy vectors have demonstrated limited utility for treatment of chronic lung diseases such as cystic fibrosis (CF). Kobinger et al. teach a vector based on a Filovirus envelope protein-pseudotyped HIV vector, which Kobinger et al. chose after systematically evaluating multiple strategies. The vector efficiently transduces intact airway epithelium from the apical surface, as demonstrated in both *in vitro* and in mouse model systems. This shows the potential of pseudotyping in expanding the utility of lentiviral vectors. Pseudotyped lentiviral vectors may hold promise for the treatment of CF (See abstract, Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nat Biotechnol.* 19(3): 225-30, 2001).

With regard to the limitation "*O*-glycosylation region within the glycoprotein has been deleted" (claims 1 and 19 of instant application), Kobinger et al. teach filovirus-pseudotyped with amphotropic envelope, Mokola envelope, EboZ envelope, Ebola-Reston (EboR) envelope, influenza-hemagglutinin (HA) envelope, and respiratory syncytial virus (RSV) F and G envelope proteins and all pseudotyped viruses were produced in parallel under the same conditions for every experiment (See Results on page 225). More specifically, Kobinger et al. teach the helper packaging construct pCMVΔR8.2, encoding for the HIV helper function, the transfer vector pHR'*LacZ* encoding for the β-gal, and plasmids encoding for envelope proteins were used for triple transfection. The transfer vector pHR'EGFP was generated by cloning the *Bam*HI/blunted *Bcl*I containing the EGFP open reading frame from pCMS-EGFP (Clontech, Palo Alto, CA) into

the *Bam*HI/blunted *Eco*RI site of pHR'*LacZ*. Plasmids encoding the following viral envelopes were used to generate pseudotyped viruses: pMD. G and pLTRMVG encoding for the Rhabdoviridae VSV-G and Mokola envelopes, pHIT 456 encoding for the oncovirus MuLV amphotropic envelope, pCB6-Ebo-GP encoding for the *Filovirus* EboZ envelope, pCB6-Ebo-GPR encoding for the EboR envelope, pSVCMVinHA encoding for the orthomyxovirus influenza-HA envelope, and pSVCMVinF and pSVCMVinG encoding for the paramyxovirus RSV F and G envelope proteins. pSVCMVinHA was engineered by cloning the blunted *Cla*I/Asp718 fragment containing the influenza envelope from BH-RCANsHA into SVCMV in at the *Sma*I site. To construct pSVCMVinF and pSVCMVinG, genomic RNA was extracted from RSV virions (American Type Culture Collection no. VR-1401) using Trizol reagent (Gibco BRL, Rockville, MD) (See Experimental protocol, page 229). Therefore, for instance, the blunted *Cla*I/Asp718 fragment containing the chimeric influenza HA envelope from BH-RCANsHA taught by Kobinger et al. read on the limitation "*O*-glycosylation region within the glycoprotein has been deleted" recited in claims 1 and 19 of instant application.

With regard to the limitation "an agent that disrupts junctions between cells" (claims 13-15, 28-30 of instant application), Kobinger et al. teach ALI culture (See Experimental protocol on page 229) and DMEM (Dulbecco's modified Eagle's medium, see second paragraph, right column, page 227). Kobinger et al. further teach that the DMEM contains 5% calf serum and 10% tryptose phosphate broth growth medium by citing the references by Dong et al. (Dong et al., A chimeric avian retrovirus containing the influenza virus hemagglutinin gene has an expanded host range. *J. Virol.* 66, 7374–7382, 1992). The DMEM medium does contain agent including sodium citrate.

Claim Rejection - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1, 4, and 19, 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kobinger et al. (Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia *in vivo*. *Nat Biotechnol.* 19(3): 225-30, 2001) taken with Feldmann et al. (Feldmann et al., The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. *Arch Virol Suppl.* 15:159-69, 1999).

Kobinger et al. teach that traditional gene therapy vectors have demonstrated limited utility for treatment of chronic lung diseases such as cystic fibrosis (CF). Kobinger et al. teach a vector based on a Filovirus envelope protein-pseudotyped HIV vector, which Kobinger et al. chose after systematically evaluating multiple strategies. The vector efficiently transduces intact airway epithelium from the apical surface, as demonstrated in both *in vitro* and in mouse model systems. This shows the potential of pseudotyping in expanding the utility of lentiviral vectors. Pseudotyped lentiviral vectors may hold promise for the treatment of CF (See abstract, Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nat Biotechnol.* 19(3): 225-30, 2001).

With regard to the limitation "*O*-glycosylation region within the glycoprotein has been deleted", Kobinger et al. teach filovirus-pseudotyped with amphotropic envelope, Mokola envelope, EboZ envelope, Ebola-Reston (EboR) envelope, influenza-hemagglutinin (HA) envelope, and respiratory syncytial virus (RSV) F and G envelope proteins and all pseudotyped viruses were produced in parallel under the same conditions for every experiment (See Results on page 225). More specifically, Kobinger et al. teach the helper packaging construct pCMVΔR8.2, encoding for the HIV helper function, the transfer vector pHR'*LacZ* encoding for the β-gal, and plasmids encoding for envelope proteins were used for triple transfection. The transfer vector pHR'EGFP was generated by cloning the *Bam*HI/blunted *Bcl*I containing the EGFP open reading frame from pCMS-EGFP (Clontech, Palo Alto, CA) into the *Bam*HI/blunted *Eco*RI site of pHR'*LacZ*. Plasmids encoding the following viral envelopes were used to generate pseudotyped viruses: pMD.G and pLTRMVG encoding for the Rhabdoviridae VSV-G and Mokola envelopes, pHIT 456 encoding for the oncovirus MuLV amphotropic envelope, pCB6-Ebo-GP encoding for the *Filovirus* EboZ envelope, pCB6-Ebo-GPR encoding for the EboR envelope, pSVCMVinHA encoding for the orthomyxovirus influenza-HA envelope, and pSVCMVinF and pSVCMVinG encoding for the paramyxovirus RSV F and G envelope proteins. pSVCMVinHA was engineered by cloning the blunted *Cla*I/Asp718 fragment containing the influenza envelope from BH-RCANsHA into SVCMV in at the *Sma*I site. To construct pSVCMVinF and pSVCMVinG, genomic RNA was extracted from RSV virions (American Type Culture Collection no. VR-1401) using Trizol reagent (Gibco BRL, Rockville, MD) (See Experimental protocol on page 229). Therefore, for instance, the blunted *Cla*I/Asp718 fragment containing the chimeric influenza HA envelope from BH-RCANsHA taught by

Kobinger et al. read on the limitation "*O*-glycosylation region within the glycoprotein has been deleted" recited in claims 1 and 19 of instant application.

With regard to the limitation "an agent that disrupts junctions between cells", Kobinger et al. teach ALI culture (See Experimental protocol on page 229) and DMEM (Dulbecco's modified Eagle's medium, see second paragraph, right column, page 227). Kobinger et al. further teach that the DMEM contains 5% calf serum and 10% tryptose phosphate broth growth medium by citing the references by Dong et al. (Dong, J., Roth, M.G. & Hunter, E. A chimeric avian retrovirus containing the influenza virus hemagglutinin gene has an expanded host range. *J. Virol.* 66, 7374–7382, 1992). The DMEM medium does contain agent including sodium citrate.

However, Kobinger et al. do not teach filovirus-pseudotyped with Marburg virus glycoprotein.

At the time the claimed invention was made, the crucial role of glycoproteins of Marburg and Ebola viruses in directing lentiviral tropism is known. For instance, Feldmann et al. teach the glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. More specifically, Feldman et al. teach that the filovirus glycoprotein plays an important role in cell tropism, spread of infection, and pathogenicity. Furthermore, Feldman et al. teach biosynthesis of the glycoprotein forming the spikes on the virion surface involves cleavage by the host cell protease furin into two disulfide linked subunits GP1 and GP2. GP1 is also shed in soluble form from infected cells. Different strains of Ebola virus show variations in the cleavability of the glycoprotein, that may account for differences in pathogenicity, as has been observed with influenza viruses and paramyxoviruses. Expression of the spike glycoprotein of Ebola virus, but

not of Marburg virus, requires transcriptional editing. Unedited GP mRNA yields the nonstructural glycoprotein sGP, which is secreted extensively from infected cells.

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention to modify the method taught by Kobinger et al. by using Marburg virus glycoprotein, by the teachings of Feldmann et al., to replace Ebola virus glycoprotein in order to have the desired surface glycoprotein on the pseudotyped lentivirus to infect targeted mammalian cells.

One having ordinary skill in the art would have been motivated to replace Ebola virus glycoprotein with Marburg virus glycoprotein to infect targeted mammalian cells because the high similarity between Ebola and Marburg viruses with regard to (i) the overall genomic nucleotide sequences, (ii) biosynthesis and processing of Ebola and Marburg virus glycoproteins, and (iii) the critical roles of Ebola and Marburg virus glycoproteins in determining the tropism of the viruses.

There would have been a reasonable expectation of success given (i) successfully performed filovirus-pseudotyped with Ebola virus glycoprotein by the teachings of Kobinger et al., and (ii) the high similarity between Ebola virus glycoprotein and Marburg virus glycoprotein by the teachings of Fedmann et al. for a skilled person in the art to pseudotype a filovirus with Marburg virus glycoprotein.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

7. Claims 1, 3, 5, 6, and 19, 23 Claims 4, and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kobinger et al. (Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia *in vivo*. *Nat Biotechnol.* 19(3): 225-30, 2001) taken with Yang et al. (Yang et al., Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med.* 6(8): 886-9, 2000).

Kobinger et al. teach that traditional gene therapy vectors have demonstrated limited utility for treatment of chronic lung diseases such as cystic fibrosis (CF). Kobinger et al. teach a vector based on a Filovirus envelope protein-pseudotyped HIV vector, which Kobinger et al. chose after systematically evaluating multiple strategies. The vector efficiently transduces intact airway epithelium from the apical surface, as demonstrated in both *in vitro* and in mouse model systems. This shows the potential of pseudotyping in expanding the utility of lentiviral vectors. Pseudotyped lentiviral vectors may hold promise for the treatment of CF (See abstract, Kobinger et al., Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. *Nat Biotechnol.* 19(3): 225-30, 2001).

With regard to the limitation "*O*-glycosylation region within the glycoprotein has been deleted", Kobinger et al. teach filovirus-pseudotyped with amphotropic envelope, Mokola envelope, EboZ envelope, Ebola-Reston (EboR) envelope, influenza-hemagglutinin (HA) envelope, and respiratory syncytial virus (RSV) F and G envelope proteins and all pseudotyped viruses were produced in parallel under the same conditions for every experiment (See Results on page 225). More specifically, Kobinger et al. teach the helper packaging construct pCMVΔR8.2, encoding for the HIV helper function, the transfer vector pHR'*LacZ* encoding for the β-gal, and plasmids encoding for envelope proteins were used for triple transfection. The

transfer vector pHR'EGFP was generated by cloning the *Bam*HI/blunted *Bcl*I containing the EGFP open reading frame from pCMS-EGFP (Clontech, Palo Alto, CA) into the *Bam*HI/blunted *Eco*RI site of pHR'*LacZ*. Plasmids encoding the following viral envelopes were used to generate pseudotyped viruses: pMD.G and pLTRMVG encoding for the Rhabdoviridae VSV-G and Mokola envelopes, pHIT 456 encoding for the oncovirus MuLV amphotropic envelope, pCB6-Ebo-GP encoding for the *Filovirus* EboZ envelope, pCB6-Ebo-GPR encoding for the EboR envelope, pSVCMVinHA encoding for the orthomyxovirus influenza-HA envelope, and pSVCMVinF and pSVCMVinG encoding for the paramyxovirus RSV F and G envelope proteins. pSVCMVinHA was engineered by cloning the blunted *Cla*I/Asp718 fragment containing the influenza envelope from BH-RCANsHA into SVCMV in at the *Sma*I site. To construct pSVCMVinF and pSVCMVinG, genomic RNA was extracted from RSV virions (American Type Culture Collection no. VR-1401) using Trizol reagent (Gibco BRL, Rockville, MD) (See Experimental protocol, page 229). Therefore, for instance, the blunted *Cla*I/Asp718 fragment containing the chimeric influenza HA envelope from BH-RCANsHA taught by Kobinger et al. read on the limitation "*O*-glycosylation region within the glycoprotein has been deleted" recited in claims 1 and 19 of instant application.

With regard to the limitation "an agent that disrupts junctions between cells", Kobinger et al. teach ALI culture (See Experimental protocol on page 229) and DMEM (Dulbecco's modified Eagle's medium, see second paragraph, right column, page 227). Kobinger et al. further teach that the DMEM contains 5% calf serum and 10% tryptose phosphate broth growth medium by citing the references by Dong et al. (Dong, J., Roth, M.G. & Hunter, E. A chimeric avian retrovirus containing the influenza virus hemagglutinin gene has an expanded host range.

J. Virol. 66, 7374–7382, 1992). The DMEM medium does contain agent including sodium citrate.

However, Kobinger et al. do not teach filovirus-pseudotyped with Ebola virus glycoprotein lacks amino acids 309-489.

At the time the claimed invention was made, the lentivirus pseudotyped with Ebola virus glycoproteins and the sequences of Ebola virus glycoproteins are known in the art. For instance, Yang et al. teach identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. More specifically, Yang et al. teach the deletion of the mucin-like region of Ebola virus glycoprotein and the deleted mucin-like region reads on amino acids 309-489.

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention to modify the method taught by Kobinger et al. by using Ebola virus glycoprotein with deleted mucin-like region, by the teachings of Yang et al., to replace the undeleted Ebola virus glycoprotein to analyze the transduction efficiency of the pseudotyped lentivirus with Ebola virus glycoprotein lacks mucin-like region.

One having ordinary skill in the art would have been motivated to replace Ebola virus glycoprotein with Ebola virus glycoprotein lacks mucin-like region because the mucin-like region encompasses the region of *O*-glycosylation, whose presence renders a low transduction efficiency of pseudotyped lentivirus expressing the Ebola virus envelope glycoprotein protein.

There would have been a reasonable expectation of success given (i) successfully performed filovirus-pseudotyped with Ebola virus glycoprotein by the teachings of Kobinger et al., and (ii) the deletion of mucin-like region of Ebola virus glycoprotein does not affect the

protein expression or function by the teachings of Yang et al. for a skilled person in the art to pseudotype a filovirus with Ebola virus glycoprotein lacks mucin-like region.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

Conclusion

8. No claim is allowed.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication from the examiner should be directed to Wu-Cheng Winston Shen whose telephone number is (571) 272-3157 and Fax number is 571-273-3157. The examiner can normally be reached on Monday through Friday from 8:00 AM to 4:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the supervisory patent examiner, Peter Paras, can be reached on (571) 272-4517. The fax number for TC 1600 is (571) 273-8300. Any inquiry of a general nature, formal matters or relating to the status of this application or proceeding should be directed to Dianiece Jacobs whose telephone number is (571) 272-0532.

PETER PARAS, JR.
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Wu-Cheng Winston Shen, Ph. D.
Patent Examiner
Art Unit 1632